

# Advance in the research of sterilants against rodents

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**Abstract:** The advances in the application of sterilizing techniques against pest rodents in China are introduced in the paper. The development of chemosterilants, improvements of botanic sterilants, production of new steroid hormone sterilants, introduction of immunostерility and excellent properties of the sterilants are outlined. The "Space Occupation Theory" of sterile techniques is advanced after practice. The botanic sterilants with gossypol and trichosanthin as its main agents were screened and successfully applied in the large area control in the northern forest area of China. The safety of sterilants to non-target animals such as rats, rabbits, dogs, monkeys and chickens was summarized.

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## Introduction

Rodent pest is one of the most serious forest disasters that cause great losses. Rodent fertility control technique is of great importance to the depression of birth rate, reduction of rodent population density and resistance of immigration based on population ecological niche, and that, it is free of environmental pollution and harmless to natural enemies and non-target animals, so it is one of the advanced techniques for "Putting prevention first, laying stress on permanent control" with brilliant future.

The pesticides, including contraceptives, spermicides, ovumcides, fetuscides and so on, used to reduce the birth rate of harmful rodents, or more exactly, acting on reproductive physiology to make one or both sexes permanently or temporarily sterile, thus lowering the amount or fecundity of filial generation are defined as rodent sterilants. It aims at lowering the birth rate of harmful rodents (Kitahara 1989).

## Research on sterilants in China

### Chemosterilants

Depressing rodent population with sterilants was taken into consideration long ago and the research on chemosterilants began quite earlier, however it has been on trial all along. At the end of 1980s, a plant in China produced "Shujuejing" with  $\alpha$ -Chloropropanol as its main agent from economic point of view, but owing to the little effect and small size of popularization, the production was abandoned. A research on sterilizing *Cricetulus migratorius*

(Pallas) with  $\alpha$ -Chlorhydrin was conducted in the end of 1990s, which was not ideal because of poor palatability. The mortality was less than 10% and sterile individuals were not more than 50% at the dosages of 200-2400 mg/kg (Liao et al. 2001; Zhang et al. 1997). Other kinds of chemosterilants acting on females are steroid and non-steroid hormone compounds terminating pregnancy and inhibiting implantation of zygotes in wombs, in other words, they are aborticides. Compounds sterilizing males are Triethylene melamine, 1,4-Butanediol dimethylsulfonate and heterocyclic compounds such as Furadantin. Ten mg/kg Hexoestrol dioctanoate baits can make both sexes sterile. The above sterilants are still on trial (Chen 1991).

In the research, it is found out that the sublethal dose of some chemical rodenticides can sterilize rodents of both sexes to a certain extent. The experiment of anti-fertility effect of Diphacine on the rats shows that compared with control group, the live fetus rate of the groups at doses of 5.00, 2.50 and 1.25 mg/kg body weight was greatly decreased; The half effective dose ( $ED^{50}$ ) fatal to fetus was measured to be  $1.602 \pm 0.676$  mg/kg body weight; The morphological observations indicated that Diphacine-Na caused testicular lesion in contrast with that of the control group. Spermatozoa, spermatid and spermatocytes in seminiferous tubules were mainly damaged. Meanwhile the wall of seminiferous tubules became atrophy, deformational and thin obviously. This implies Diphacine-Na has anti-fertility effect on both male and female rats. The control effect is obviously superior to that of the simple killing (Xu et al. 2001).

### Botanic sterilants

It is shown in the experiments that dozens of natural plants and their extracts are sterilizing. Matters anti-implanting and abortive are as follows. Embelin, arisofolic acid methylester, m-dimethylhydroquinone, Paraguay

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chrysantheminol, lignitol, sparteine, trichosanthin, terpene ortho-ester dibasic, Yuanhua cinum and Yuanhua dinum are strongly abortive. Protopine, Corynoline and Isocorynoline can terminate early pregnancy while Colchicina extracted from plants of Liliaceae inhibits the production of sperms. The alcohol extract of *Curcuma zedoaria* (Berg.) Rosc., compounds of terpene and sesquiterpene are obviously anti-pregnant.

In early 1980s, some experts in China began their research on botanical sterilants. The result of anti-fertility experiments against rats with cottonseed showed that sperms in seminal ducts and caudal epididymis became dead, less in quantity and deformational; Seminiferous tubules of some rats were partial retrograde and the production of sperms was inhibited (Dai et al. 1980). Gossypol, being the only anti-fertility component in cottonseeds, is toxic to animals. Its toxicity is correlated with application dosages. Small dose of gossypol has an anti-fertility effect with no severe impact on environment and non-target animals (Dai et al. 1978). Cottonseed obviously interferes the amino acid metabolism of testes when applied on adult mice for 15 consecutive days. The lowest effective doses are 100:10-00:15. The effective date is 15 days after the treatment. The interference of cottonseed in amino acid metabolism of testes had certain impact on the normal physiological function of testes (Chen et al. 1986).

Raw trichosanthin also influences the amino acid metabolism of ovaries of female mice and distinctly changes the content of amino acids in ovaries, especially the reduction of amino acids composing histone interferes the development of ova and the lasting time of corpus luteum. The 100 : 10 dose group reacts distinctly to raw trichosanthin in the aspect of amino acid metabolism of ovaries and amino acids that make up histidine or have aromatic rings increased 10 days after the treatment. Raw trichosanthin injures trophoblast cells, too. The degree of injury is positively correlated to the dosage. The cells reveals high susceptibility to raw trichosanthin (Xiong et al. 1976; 1981).

The experiment administrating gossypol to rats showed that the speed of gossypol accumulation in vivo is correlated with the oral dosage, the bigger the dosages, the higher the concentration of gossypol in the liver and blood is. The absorption is in direct proportion to dosage. The toxicity of bigger dosages can't be neglected although the dosages can achieve the anti-fertility affect earlier. So smaller dosage (20 mg/d for each rat) and certain duration of administration is suitable (Ke et al. 1979). The anti-fertility way of gossypol proved to be its function on spermatoblasts in testes to destroy the structure of respiratory chains on the mitochondria cristal membrane in cells resulting in the uncoupling of oxidative phosphorylation, interference of ATP synthesis of sperms and sharply reduction of ATP content. Owing to the sustained effect of gossypol, the destruction of structure of respiratory chains became increasingly serious and revealed itself in ultrastructure, finally sperms died of energy deficiency (Ke

1982).

The experiments were made on the use of gossypol against rats, mice, buff-breasted rats (*Rattus flavipectus* Milne-Edwards) and Norway rats (*Rattus norvegicus* Berkenhout). The result showed it had killing effect (Lin et al. 1988; Chen et al. 1985). Trials with gossypol acetic acid against *Myospalax fontanieri* Milne-Edwards were carried out in Manma Grassland, Xiahe County, Gansu Province. The result showed that the eaten rate of gossypol baits by *Myospalax fontanieri* was 85.21%. *Myospalax fontanieri* were captured in the experimental area for sperm survival examination on the spot 7 days after the treatment, the survival rate of sperms was 14.35%. It implies that the influence of gossypol on sperms of *Myospalax fontanieri* is obvious. Ten days after the application, examination was made on the rats captured on the spot. The result showed the pregnant rate of gossypol-eaten female rats was 56.25% while that of the rats who didn't eat the gossypol was 81.82%. Investigation on the number of mound groups showed no sharply reduction of *Myospalax fontanieri* population in a short time after the application of gossypol against *Myospalax fontanieri* (Yan et al. 1990).

Studies on the mechanism and application techniques of botanic sterilants were made and achieved significant breakthrough in early 1990s when the first generation of botanic sterilant with gossypol and trichosanthin as its main agents was screened out against *Clethrionomys rufocanus* Sundevall and *Apodemus speciosus* Temminck and successfully popularized in the northern provinces of China in an area of 28 000 hm<sup>2</sup> or more (Zhongguo Linye Bao 1993; Zhao, et al. 1994).

Toxicological research on botanic sterilants against wild rats showed (1) botanic sterilants caused the increase of albumin content in blood plasma and the reduction of  $\alpha$ -globulins and  $\beta$ -globulins, thus seriously hindered the transfer and transportation of sex hormone in the course of reproduction (Zhang et al. 1994a); (2) their influence on amino-acid in genital tissues found expression in reducing the amount of amino-acid in testicle and epididymis tissues; (3) their destruction on spermatogenous tissues of male testicles expressed in the fall-out of most spermatogenous epithelial cells of testicle seminiferous tubules, of which spermatocytes and spermatoblasts disappeared. Ghosts and nucleus-splitting cells could be found in the cavity of tubules. The nuclei of surviving spermatocytes in the lower part of spermatogonia were pyknotic and margins between cells indistinct. No sperms were found in the cavity of tubules and seminiferous tubules atrophied (Zhang et al. 1994b).

### Improved botanic sterilants

The improved sterilant with Zedoary turmeric powder added into the original main agent was developed in late 1990s, thus heightened its function on female sterilization. The result of the experiments against *Clethrionomys rutilus* Pallas, *Apodemus agrarius* Pallas, *Microtus fortis* Buchner,

*M. maximowiczii* Schrenck and *Myospalax myospalax* Laxmann with the improved sterilant showed that it had good effect on female sterilization of harmful rats. The pregnant rate declined compared with that of control groups. And at the same time the fetuses of controls were in late embryonic developmental stage while most fetuses of experimental rodent in initial and mid developmental stages. The sterilant could impede the embryonic development. Uterine oedema and blood-spill were found in most of the experimental female rats and caused abortion and dead fetuses. The sterilant functioned strongly in destroying trophoblast cells in uterine placentae and blocking the nutrition transportation channel from mother to fetus, and finally resulted in sterilization. The sterilant mainly acts on pregnant rats besides its sterilizing function on male.

#### **Sterilant with sex hormone as its main agent**

At the same time, the 2<sup>nd</sup> generation of sterilants, a mixture of synthetic steroid hormone & gossypol, was developed to sterilize forest rodents. It mainly destroys the testicle---a reproductive organ of male and inhibits ovulation of female. It destroys and affects the development, formation and ovulation of normal reproductive cells (sperms and ova), which are regulated by estrogen and progestogen. The catch rate in the experimental plot was 1.33%-2.33% in October of the year while it was 8.33%-10.33% in the control plot, which was 3 or 4 times that in experimental plot. The population density kept low in the experimental plot up to October the next year when the catch rate was 3%-4%. So the interference of synthetic steroid hormone sterilant is successful and anti-fertility effect is obvious. It's long lasting and stable (Yang 1999). Detailed research was also made on the effect of Ma gossypol sterilant on the population density, propagation and age structure of *Clethionomys rufocanus*, *Apodemus speciosus* and the safety of rodent natural enemies and non-target animals.

#### **"Space Occupation Theory" of sterile techniques**

"Space Occupation Theory" was summarized from several-years' investigations. Controlling pest rodents with sterilants wouldn't kill individual harmful rodent. It only lower the reproduction ability and population density by control the rodent's fertility. In the treated areas, the harmful rodents are still alive, but the age structure of the population is of a proportion in favor of old and mature rats, tending to be declining. The old and mature rats live in the treated area, occupy some food resource and living space and compete with the rats moving in from adjacent area. The emigrants and immigrants are dynamically balanced. The unharmed natural enemies of rats restrain and control the rodent's population density. The above factors keep the population density in a low level and maintain it for a period, realizing the aim that there are some rodents in the treated forest but cause no plague. In fact, the sterile technique is ecological measures for rodent control (Yang *et al.* 1991).

#### **Introduction and experiment of immunosterility techniques**

In the U.S, the potential genetic control methods against pest rodents were studied. That is to treat male individuals in the natural population with  $\times$  and  $\delta$  rays and make them produce sperms with sterile genes, then release them to mate after competition with other males in the population. Although the gene order of rats is complex and difficult for artificial mutagenicity, progress has been made (Liu *et al.* 1999)

Along with the appearance of immune technique--a new advance of research on human contraception, rodent sterility progressed. Immunosterility means the link of polypeptide or protein regulating hormone of rodents with immunocompetent fragments or other foreign macromolecular matters to form antigens. When they enter the body of animal, it produces antibody destroying the reproductive-regulating hormones in it, thus blocking fertility (Wang, *et al.* 1996).

Foreign scientists explored the production of recombinant vaccines by means of gene engineering and they succeeded in developing several kinds of sterile vaccines, which could make pest rodents sterile all their lives (Millar *et al.* 1989 Miller 1993). In addition, a 385-base pair complementary DNA and its genomic DNA named Bin 1b was cloned in the male reproductive system of rats, which appeared to have an important role in reproductive functions as sperm-immobilizing activity and cytotoxicity for mouse oocytes and preimplantation embryos. Sterile vaccines are proteins. Their antibodies could significantly reduce the sex hormone in both sexes. For a few vaccines, a very small amount can reach the aim of sterility. So its anti-fertility effect is excellent. But the production of the vaccines requires high technology of gene recombination and molecular biology, so it is very expensive. At present it is only introduced into institutes and difficult for the application in agricultural and forestry practice. The popularization of this sterile technique has to wait long.

#### **Safety of sterilants**

##### **Safety to rats**

The toxicity of gossypol on rats is as follows: the median lethal dose ( $LD_{50}$ ) of pure gossypol on rat orally is 2630 mg/kg body weight for aqueous solution, 2325 mg/kg for oil solution. Small doses (15-30 mg/kg) of gossypol were given to rats orally for a long time, no obvious morphological changes in viscera of rats such as hearts, livers, spleens, lungs and kidneys were observed (Ke 1982).

##### **Safety to rabbits**

It's proved that gossypol is toxic to rabbits. Rabbits weighing 4 pounds lost their appetite, but no poisoning symptoms were found when they were given 0.5g gossypol

orally. Adding 225-450  $\mu\text{L/L}$  gossypol into rabbits' diet, two rabbits lost their appetite and had a spasmotic paralysis in hind legs 2 months after the administration of gossypol. The phenomenon occurred also in domestic cats. Rabbits given 10 mg gossypol /kg body weight for 219 days didn't die (Ke 1982).

### Safety to dogs

Dogs are very sensitive to gossypol. Experiment was made on acute toxicity (30 mg/kg), subacute toxicity (3 mg/kg) and chronic toxicity (1.5 mg/kg) of gossypol on dogs. Twenty dogs were divided into 5 groups. The result showed 3 dogs died respectively on the 24th, 25th and 28th days after the administration and the fourth dog withdrew 30 days after administration in the group for acute toxicity; 4 dogs died respectively on the 43rd, 51st, 62nd and 64th days in the subacute toxicity group; one dog died suddenly on the 60th day after the administration in the chronic toxicity group. The examination showed there were pathological changes in hearts, livers, kidneys and lungs of dogs in acute and subacute toxicity groups while there were no obvious changes in viscera of the dog in chronic toxicity group (Ke 1982 ).

### Safety to monkeys

Monkeys were given gossypol orally 2-4 mg/kg (body weight) daily for 3 consecutive months, then withdrew one and a half months. After that, they resumed the administration for another 3 months. The result showed the appetite and activity of monkeys were normal, the weight increased, and no other influence except the small changes in electrocardiogram. Study on the chronic toxicity of gossypol on monkeys indicated no obvious changes in electrocardiogram, kidney function, electrolytic metabolism and sexual function. There was no abnormality in hemogram, carbon dioxide combining power and chest roentgenoscopy before or after the administration (Ke 1982).

### Safety to chickens

Experiment was made in the General Station of Forest Pest Management, the State Forestry Administration. The result showed oral administration of gossypol-trichosanthin compound didn't influence the production of eggs, the hatch rate and copulating ability of cocks, but had some impact on the body weight of chickens when the gossypol content in the diet reached 0.016% or more. Gossypol- $^{14}\text{C}$  had little influence on laying hens (Albon-Donia *et al.* 1970).

The advantages of sterilizing techniques are as follows: (1) effective control of pest rodent population for a long time; (2) free of environmental pollution, safe to natural enemies of rodents and non-target animals; (3) a countermeasure for permanent control from the ecological and biological point of view. As the technique is improving day by day, the prospect of controlling forest rodent pest with sterilizing technique is wide.

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